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Mycobacterium avium: an Emerging Pathogen for Dog Breeds with Hereditary Immunodeficiencies

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Abstract

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Summary Given the zoonotic potential of a MAC-infected dog particularly to immunocompromised human patients, diseased dogs pose a public health risk. While not a reportable disease, treatment of systemic mycobacteriosis is generally not effective and discouraged in dogs. The collaborative efforts by microbiologists, veterinary clinicians, dog breeders, primary care physicians, and infectious disease specialists applying the One Health approach are therefore crucial for the best management and prevention of MAC infection.

Keywords *Mycobacterium avium* · Dog · Non-tuberculous mycobacteria · Precision medicine · Cutaneous and disseminated mycobacteriosis

Introduction

Mycobacterial infections are caused by bacteria that belong to the family *Mycobacteriaceae*, order Actinomycetales. The genus *Mycobacterium* recently underwent important taxonomic change proposals, and the redistribution of 150 *Mycobacterium* species into five new genera has become a controversial issue among microbiologists, clinicians, and researchers [1, 2•]. Besides the emended genus *Mycobacterium*, the four newly proposed genera *Mycobacteroides*, *Mycolicibacter*, *Mycolicibacterium*, and *Mycolicibacillus* are still debated regarding the phylogeny and taxonomy of large bacterial clades. On one hand, the necessity of an up-to-date core genome sequence-based phylogeny and taxonomy of a large heterogeneous group, such as the genus *Mycobacterium*, contributes to a more precise understanding of bacterial rise and evolution [1]. On the other hand, such drastic changes have led to confusions and potentially impede the exchange of information between researchers, microbiologists, and clinicians and, thereby, to patients and pet owners [2•, 3]. Moreover, some of the newly proposed genera or families comprise pathogens causing various notifiable diseases worldwide; therefore, misinterpretation of microbiology test results may occur. Few species mentioned, hereafter, have been recently published according to the newly proposed nomenclature, e.g., *Mycolicibacterium smegmatis* for *Mycobacterium smegmatis*. Therefore, for the sake of clarity, mycobacterial species in the present manuscript will be preceded by "*M.*" referring to *Mycobacterium*, regardless of the recently proposed nomenclature.

Although extremely heterogeneous in their host affinity and pathogenic potential, mycobacteria share important morphological similarities. Mycobacteria are aerobic bacteria,

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non-spore-forming, and non-motile. Because of the high concentration of mycolic acid in their cell walls, mycobacteria are difficult to stain with common techniques, such as the Gram stain. However, once mycobacteria are heated in the presence of carbol-fuchsin, this stain can penetrate their lipid-rich cell wall. And while other non-acid-fast bacteria are commonly decolorized by acid alcohol, mycobacteria are able to retain the Ziehl-Neelsen dye [4]. For this reason, mycobacteria are also commonly called acid-fast bacteria, which is, however, not unique to this genus. Other medical-relevant genera belonging to the Actinomycetales, such as *Nocardia* and *Rhodococcus*, are also acid-fast and could be erroneously misidentified as mycobacteria by Ziehl-Neelsen stain.

The recent enormous advances in molecular genetic techniques allowed readily the sequencing of single or multiple housekeeping genes followed by whole genome sequencing of any bacteria. Microbial isolates can nowadays be identified with the highest accuracy expediently and relatively inexpensively which led to the definitive description and partial reclassification of a myriad of new species and genera as well as precision medicine. Alone during the past decade, more than 50 new species of mycobacteria were published [5].

Although whole genome sequencing of bacteria is being progressively implemented in diagnostic laboratories, it is not (yet) feasible for daily routine diagnostic purposes. Currently, reverse hybridization-based line-probe commercial kit assays are commonly used for mycobacterial identification of clinical isolates. In addition, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has proven to be a rapid and accurate tool in the diagnostic workflow of larger laboratories [6, 7]. Nevertheless, accuracy of the MALDI-TOF MS-based species identification remains inferior to genome sequencing [5].

Mycobacterial diseases are clinicopathologically divided into two main groups: tuberculosis and mycobacteriosis.

Tuberculosis (TB) in humans and different animal species is caused by the members of the *Mycobacterium tuberculosis* complex (MTBC), which are obligate pathogens showing marked host predilections. The MTBC includes currently the human-adapted species *M. tuberculosis* and *M. africanum*; the animal-adapted *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. orygis*, the "dassie bacillus"; and the more recently discovered *M. mungi*, the "chimpanzee bacillus", and *M. suricattae* [8]. In addition, *M. canettii* is also considered a member of the MTBC based on nucleotide identity, albeit opportunistic infections in humans [8]. The members of the MTBC are believed to be host-adapted and, with the exception of *M. canettii* which has a putative environmental reservoir, they can be found in contaminated environments. Dogs infected with *M. tuberculosis*, *M. bovis*, and *M. microti* have been rarely documented worldwide [9–11]. Therefore, dogs are considered a spillover host for MTBC pathogens, meaning that there is no maintenance of the pathogen within the canine population without continued exposure to a primary host and reservoir [12].

The modality of infection with MTBC members depends on the specific pathogen (*M. tuberculosis*, *M. bovis*, *M. microti*) and the geographical and socio-economic environment of dogs. The infection with the human-adapted *M. tuberculosis* is spread almost exclusively by airborne transmission, and the lungs are its primary target. The mycobacteria can disseminate, resulting in various other clinical manifestations depending on the affected organ. To the contrary, *M. bovis* and *M. microti* are believed to be transmitted to dogs via contaminated animal products. Independently from the infecting species, canine TB is characterized by a long incubation period (months to years) resulting in asymptomatic carriers and chronic slowly progressive disease that can be often detected solely by indirect diagnostic test methods.

In most countries, contrary to human and livestock species, collection and submission of samples for mycobacterial testing from a dog when suspecting TB is not mandatory, but rather initiated based upon good clinical judgment by a clinician and dog owner. Moreover, there are no regulations on managing dogs after documentation of MTBC, but due to the high zoonotic risk, euthanasia is generally recommended. Furthermore, the prolonged administration of antituberculosis agents can be highly toxic to dogs.

Mycobacteriosis is caused by opportunistic pathogenic non-tuberculous mycobacteria (NTM). The NTM are ubiquitous, e.g., in soil and water, and healthy humans and animals are considered fairly resistant to mycobacteriosis. Among NTM, two species are recognized as true pathogens for humans, namely *M. marinum* and *M. ulcerans* [13]. Nevertheless, more than 60 species of NTM are known to be opportunistic pathogenic to humans and other mammals, and infections with these emerging pathogens are now more common than tuberculosis in industrialized countries [5, 14, 15]. Geographical differences concerning the prevalence of different NTM have been described in veterinary and human medicine, e.g., higher prevalence of feline leprosy caused by a number of mycobacteria, namely, *M. lepraemurium*, *M. visibile*, and novel mycobacterial species such as *Mycobacterium* sp. Tarwin, in cats is seen in temperate maritime climates [16]. Similarly, the buruli ulcer, a chronic skin disease in humans caused by *M. ulcerans*, occurs mostly in tropical and subtropical regions [17]. The most common NTM species encountered in humans with lung infections are members of the *Mycobacterium avium* complex (MAC), *M. abscessus*, and *M. kansasii* depending on geographical regions [18]. Numerous NTM have been reported in dogs causing a variety of clinical manifestations, including skin, respiratory, gastrointestinal, and disseminated disease.

To date, the virulence of specific mycobacterial species and their strains for a particular host and whether a transmission

from domestic or wild animals to humans and vice versa can occur remain largely unknown. Moreover, the reason why ubiquitous mycobacteria are able to cause serious life-threatening illness to some individuals while being innocuous to other individuals of the same species is now being elucidated. In human and veterinary medicine, underlying hereditary and acquired immunodeficiency disorders have recently been discovered to cause predispositions specifically to mycobacteriosis and may guide selection of treatment including triple drug therapy. In dogs, infections due to NTM are sporadically reported worldwide, but the exact source of the mycobacteria remains in most cases unknown. Epidemiological data concerning NTM infections of dogs are missing. Moreover, time-consuming and laborious detection methods and a low disease awareness could be responsible for a potentially large number of undiagnosed cases. Indeed some dogs in Miniature Schnauzer breed have a common genetic predisposition to NTM which has been documented from clinicopathological signs to the molecular genetic defect. A rapid genotyping approach currently permits precise detection of diseased dogs and asymptomatic carriers, facilitating specific management and prevention in future generations.

Mycobacterium tuberculosis

Mycobacterium tuberculosis is the primary cause of TB in humans worldwide, with the exception of few specific geographical regions (e.g., *M. africanum* in West Africa), and can also occur in dogs after prolonged close contact with TB patients [19, 20]. *Mycobacterium tuberculosis* can be experimentally transmitted by close contact between animals, and, consequently, infected dogs could act as reservoir for human infections [21]. Pulmonary, cutaneous, and disseminated disease manifestations have been reported in dogs [11, 22–25]. In most cases, the infection develops with granulomatous inflammation localized mainly to the lungs, skin, kidneys, bone marrow, and/or digestive system and associated lymph nodes [21].

Mycobacterium bovis

Mycobacterium bovis is the pathogenic agent causing TB with the broadest known host spectrum. Infection chains in numerous domestic and wild mammal and the resulting transmissions to humans have recently led to the term "zoonotic TB" by the World Health Organization (WHO) [26]. Furthermore, the number of *M. bovis*, and to a lesser extent by the closely related *M. caprae*, infections in humans might well be underestimated because of a lack of diagnostic surveillance programs [26]. Nevertheless, *M. bovis* infections are rare in dogs and, as in humans, mostly associated with consumption of raw milk or contaminated byproducts from affected ruminants [27–29]. Clinical findings of infected dogs vary from respiratory signs and cutaneous lesions to gastrointestinal signs accompanied by fever, inappetence, and weight loss.

Gray wolves (*Canis lupus*), ancestors of the domestic dog, are susceptible to natural infections with *M. bovis* and *M. caprae* [30, 31] and may be infected by their preys such as domestic and wild ruminants. Interestingly, their TB seems to be mild and self-limiting. Since diseased wild ungulates are an easy prey of wolves, they may play a role in controlling TB in wild ruminant populations [31]. Domestic dogs may be more prone to tuberculosis because of their restricted genetic pool and associated increased inbreeding practices.

Non-tuberculous Mycobacteria

Non-tuberculous mycobacteria (NTM) are commonly divided into rapidly growing mycobacteria which usually grow in subculture within 1 week and those that require longer incubation to form visible colonies and are termed slowly growing mycobacteria such as the members of the MAC [15]. In dogs, a large variety of rapid- and slow-growing mycobacteria can cause mycobacterial granulomas of the cutis and subcutis, and the resulting lesions are clinically mostly indistinguishable [32••].

The *Mycobacterium* sp. causing canine leproid granulomas has yet to be isolated in culture and sequenced. Canine leproid granuloma is a nodular dermatosis affecting the cutis and subcutis of the dog, usually located on the head, especially on the dorsal fold of the ears. Although the geographical distribution of the disease is probably wider, dogs with leprosy have been mostly described from Australia, where it is the predominant mycobacterial disease of dogs, Brazil and the USA, particularly in the Central Valley of California [32••, 33, 34].

Mycobacterium ulcerans, the environmental pathogen responsible for the Buruli ulcer disease, can infect both humans and animals and remains a major public health problem [17, 35]. Although the ecology and transmission of this pathogen remain mostly unexplained, recent research and phylogenetic analyses suggest a common environmental source of contamination for humans and animals, including domestic dogs [35]. Painless nodules, papules, necrotic ulcers, plaques, and/or edema of the cutis and subcutis are common clinical features of this infectious disease in humans and dogs [36]. In addition, similar chronic non-healing cutaneous and subcutaneous lesions have been described in dogs with rapidly growing (e.g., *M. smegmatis* [37], *M. goodii* [37, 38], *M. fortuitum* [37, 39], and *M. chelonae-abscessus* [40]) and slowly growing mycobacteria, (e.g., MAC, *M. nebraskense* [41]).

Apart from cutaneous and subcutaneous infections and the subsequently discussed MAC infections, clinical manifestations due to NTM infections in dogs have been rarely reported, e.g., pulmonary disease due to *M. fortuitum* [42–44], mastitis and persistent pleural effusion caused by *M. kansasii* [45, 46], or disseminated disease characterized by lameness and ataxia with *M. smegmatis* [47] and *M. genavense* [48].

Mycobacterium avium Complex

The MAC, encompasses a heterogeneous group of slow-growing NTM that are opportunistic pathogenic for humans and animals. Historically, the complex consisted of two species: *M. avium* and *M. intracellulare.* This classification was based upon the pathogenity to birds, being *M. avium* pathogenic and *M. intracellulare* experimentally avirulent to fowls [49••]. With the major diagnostic improvements and heightened awareness among clinicians, new MAC species were described including *M. arosiense*, *M. bouchedurhonense*, *M. chimaera*, *M. colombiense*, *M. ituriense*, *M. lepraemurium*, *M. marseillense*, *M. paraintracellulare*, *M. scrofulaceum*, *M. timonense*, *M. vulneris*, and *M. yongonense* [49••]. The frequency of isolating MAC members varies geographically with *M. intracellulare* seen frequently in human patients in Australia and South Africa and *M. avium* predominating in Europe and the Americas [50]. In immunocompromised humans, such as HIV patients or transplant recipients, MAC is the leading cause of pulmonary and disseminated mycobacteriosis [15, 49••]. However, healthy humans and animals are considered to be rather resistant to develop MAC-related mycobacteriosis.

The species *Mycobacterium avium* is currently divided into four subspecies including *avium* (MAA), *silvaticum* (MAS), *hominissuis* (MAH), and *paratuberculosis* (MAP) based on phenotypic peculiarities such as mycobactin J dependency (MAP and MAS) or specific genetic markers that will be further discussed below [49••, 51••]. It has to be mentioned that, because of financial or technical constraints, only specialized laboratories can accurately distinguish between MAA, MAH, or novel MAC members, such as *M. colombiense* and *M. chimaera*. As indicated by its name, the subspecies MAH is frequently recovered from humans and swine. In addition, this ubiquitous environmental saprophyte has a broad host range, and cases of disseminated MAH infection have been described in other mammals, including dogs, cats, cattle, goats, domestic rabbits, free-ranging red deer, African elephants, and horses [52–59].

Remarkable genetic heterogeneity among MAH strains has been elucidated with specific molecular markers including internal transcribed spacer (e.g., sequevars Mav-A and Mav-B), insertion sequences (IS*Mav6*), or multispacer sequence typing (e.g., MST22) [60•, 61•, 62]. Consequently, an association between certain genotypes and increased virulence, ability of long-term persistence and biofilm formation, or host-species predilection is evident.

Assuming that MAP is an obligate pathogen of ruminants and MAA/MAS are obligate pathogens of birds, MAH has been proposed to be the only true environmental subspecies of *M. avium* [51••]. MAP is an obligate intracellular pathogen, especially in ruminant species and is the etiological agent of paratuberculosis, a contagious disease listed by the World Organisation for Animal Health and also known as Johne's disease. This chronic progressive intestinal disease has also been reported in horses, pigs, deer, alpaca, llama, rabbits, free-ranging carnivores, and dogs [52, 63, 64]. The granulomatous gastroenteritis is causing diarrhea, malabsorption, decreased milk production, and wasting [52, 64, 65]. The MAA and *M. genavense* are considered a major bird pathogen, causing avian mycobacteriosis but only occasionally infections and disease in other animals and humans [51••]. In contrast, MAH has only rarely been isolated from tuberculous lesions in birds [66]. Therefore, the common belief that contaminated poultry carcasses or excrements/droppings are a major source of MAC infections for dogs should be dismissed.

In most published canine case reports, the involved *M. avium* subspecies was not identified [67–76]. However, according to recent studies and unpublished data by authors, MAH appears to be the predominant pathogenic subspecies [77•, 78•, 79•, 80•]. Occasionally, mycobacteriosis due to MAP [64] or MAA [81] has been reported in dogs. To date, there are over 43 published cases reporting MAC-related mycobacteriosis in dogs (Table 1); however, there are many more unreported cases particularly in the Miniature Schnauzer and Basset hound breeds. Absence of epidemiological data for a non-notifiable disease, negligible impact of additional case reports, and misdiagnoses are some of the reasons for this underreporting.

As MAH is ubiquitous and dogs are likely confronted with this opportunistic pathogen from an early stage of their life almost daily, it can be stated that the number of clinical infections caused in dogs and humans is extremely low. However, as it is for humans, immunocompromised dogs can develop systemic and life-threatening mycobacteriosis due to MAC infection. And indeed even among the published case reports (43 cases), a breed predilection to MAC infection is evident for Miniature Schnauzers (16 cases) and Basset Hounds (10 cases). Because over a half (60%) of all published cases are in breeds with a known genetic predisposition, the summary of these cases is heavily influenced by those with a clear breed predisposition. Among the case reports, most affected dogs were young, with the highest risk of developing signs within the first 3 years of age and only rarely in dogs older than 5 years. Weight loss, lethargy, and inappetence were the predominating signs, followed by lymphadenopathy, splenomegaly, and gastrointestinal signs. Other less common clinical signs included spinal pain, paresis, lameness, subcutaneous swellings, and diffuse alopecia. Extensive granulomatous invasion was found in various examined organs including the small and large intestine, spleen, liver, lungs, bone marrow, and various lymph nodes (inguinal, mediastinal, mesenteric, prescapular, submandibular) in most dogs.

While there are many genetic predispositions to infections in humans and animals, few are restricted to very specific organisms like mycobacteria. Hereditary immunodeficiencies in human patients with mycobacteriosis have been associated

Table 1 Signalment, country of origin, clinical findings, treatment, and outcome of 43 published cases of *Mycobacterium avium* complex infection in dogs. Although the overall number of reported cases worldwide is low, a breed predisposition for Miniature Schnauzer and Basset Hound dogs is apparent. In these breeds, many more unreported cases have occurred, and all affected Miniature Schnauzers tested are homozygous for the *CARD9* variant. Most affected dogs were young (less than 4 years) and unspecific clinical signs such as weight loss, lethargy, and generalized lymph node enlargement were common findings

Breed	Age sex	Country	Clinical findings	Detection of MAC	Antimycobacterial treatment	Outcome
Miniature Schnauzer	3 litter mates: 2 yr M/F and 3 yr M [117]	USA	Lethargy, inappetence, hematochezia, lymphadenopathy, vomiting, diarrhea	LNs, Liver, spleen, GI, BM	None	Euthanased
	2 yr FS [73]	USA	Subcutaneous swelling, respiratory difficulty	LNs, liver, spleen, GI, BM, lung	Enrofloxacin, clofazimine, ciprofloxacin, rifampin	No improvement over 6 weeks, euthanased
	8 dogs from different breeders M = 5/F = 3 1 yr = 2/2 yr = 4/3 yr = 2 [118]	Argentina	Lethargy, inappetence, lymphadenopathy, vomiting, diarrhea	LNs	None	Died within short time or euthanased
	2 yr M [67]	Germany	Anorexia, lymphadenopathy, hematochezia, diarrhea	LNs, lung, spleen, liver, GI, thymus, adrenal glands, BM, myocardium	Several unknown antibiotics	Short-term improvement, euthanased
	3 yr [78•]	Germany	Lethargy, weight loss, lymphadenomegaly, diarrhea	LNs, lung	Unknown	Euthanased
	2 yr F [119]	South Korea	Stillbirth, anorexia, lymphadenopathy	LNs, spleen	Doxycycline, clarithromycin 3 months	Successful treatment
	2.5 yr M[§]	Switzerland	Lethargy, inappetence, lymphadenopathy, vomiting	LNs, liver, spleen	None	Euthanased
Basset Hound	5 dogs < 5 yr [120] M = 3/F = 1/FS = 1	USA	Inappetence, lameness, vomiting, diarrhea, thoracolumbar pain	LNs, GI, lung, spleen, liver, CNS, kidney, thymus, BM, bile duct, adrenal gland, tricuspid valve	Amoxycillin, ethambutol, isoniazid, streptomycin, trimethoprim/-sulfamethoxazole	No improvement, euthanased
	3 yr FS [75]	USA	Anorexia, lymphadenopathy shivering	LNs, spleen, liver, BM, lung, kidney	Isoniazid	Continued deterioration over 10 m, euthanased
	4 yr M [121]	USA	Weight loss, diarrhea	LNs, spleen, liver, BM, GI, lungs	None	Euthanased
	2 yr F [77•]	Italy	Lymphadenopathy, lameness, diffuse alopecia	LN, spleen, liver, BM, lungs	None	Euthanased
	Adult M [68]	Italy	Anorexia, lymphadenopathy	LNs, Liver	Enrofloxacin, rifampin, clarithromycin	No improvement, euthanased
	3.5 yr M [72]	Greece	Anorexia, diarrhea, splenomegaly, lymphadenomegaly	LNs	Unknown antibiotics	No improvement, euthanased
Staffordshire Bull Terrier	3 yr M [122]	UK	Weight loss, vomiting, diarrhea	LNs, liver, kidney, prostate, lung	None	Euthanased
	4 yr M [76]	South Africa	Depression, weight loss, uveitis	LNs, spleen, liver, kidney, BM	Doxycycline 1 month	Short-term improvement, euthanased

Table 1 (continued)

Breed	Age sex	Country	Clinical findings	Detection of MAC	Antimycobacterial treatment	Outcome
Labrador Retriever	4 yr M [70]	Canada	Lameness	LNs, spleen, liver, GI, kidney, adrenal gland, lung, CNS, BM,	None	Euthanased
	10 months [123]	New Zealand	Anemia, lymphadenopathy, splenomegaly	/	/	/
Great Pyrenees	4 yr FS [124]	USA	Weight loss, anemia, diarrhea, lameness	Blood, LNs, spleen, liver, lung, GI, adrenal cortices, BM	Amikacin	Euthanased
Australian Terrier	12 yr MC [80•]	Australia	Weight loss, lymphadenomegaly, diarrhea,	LNs, spleen, liver	Rifampicin, clarithromcyin, moxifloxacin, doxycycline 52 weeks	Prolonged treatment (79 weeks) euthanased
Golden Retriever	3 yr MC [98]	UK	Progressive nasal swelling, lymphadenopathy	Nasal mass	Enrofloxacin, clarithromycin, rifampicin 9 months	Successful treatment
Poodle	3 yr FS [125]	South Korea	Lethargy, anorexia, splenomegaly, lymphadenomegaly, skin disease	LN, spleen	Enrofloxacin, clavulanate/amoxicillin, doxycycline, rifampicin	No improvement, euthanased
Australian Shepherd	3 yr MC [97]	USA	Multiple subcutaneous nodules, lymphadenopathy	Skin	Rifampicin, clarithromycin 4 months	Successful treatment
Shih Tzu-Poodle-cross	2 yr MC [74]	USA	Lethargy, anemia, abdominal pain, lymphadenomegaly, splenomegaly	LNs, spleen, liver, lung, GIT, thymus	Enrofloxacin 2 months	No improvement, euthanased
Maltese-cross	3 yr MC [71]	Australia	Diarrhea, hematochezia, lymphadenomegaly	GI	Cephalexin, metronidazole, enrofloxacin	No improvement, euthanased
Yorkshire Terrier	1 yr [78•]	Germany	Lethargy, weight loss, lymphadenomegaly, diarrhea	LNs, lung	Unknown	Euthanased
German Elo	9 yr F [79•]	Germany	Lethargy, PU/PD, weight loss, pruritic skin disease	Skin	Doxycycline/ metronidazole; rifampicin/ pradofloxacin, 4 months	Successful treatment
Crossbreed	4 yr FS [69]	France	Anorexia, PU/PD, lymphadenomegaly, diarrhea	Blood, LNs, spleen, liver, lung, GI	Unknown antibiotics	No improvement, euthanased
	5 yr FS [126]	USA	Hematochezia, diarrhea	LNs, spleen, liver, GI	Ampicillin	Short-term improvement, euthanased
	Young adult F [127]	USA	Hindlimb paralysis, proprioceptive deficits	Spinal cord, adrenal glan	None	Euthanased
	1.5 yr F [81]	Italy	weight loss, lymphadenomegaly	LNs, BM	Pradofloxacin, azithromycin, rifampicin	Died during therapy

PU/PD polyuria/polydipsia, *BM* bone marrow, *GI* gastrointestinal tract, *LN(s)* lymph node(s), *CNS* central nervous system, *M* male, *MC* male castrated, *F* female, *FS* female spayed

§ Ghielmetti unpublished

with variants in both subunits of the IFN-γ receptor, beta-1 chain of the interleukin-12 receptor, or the signal transducer and activator of transcription 1 (STAT1), which plays a crucial role in controlling intracellular responses to bacteria and viruses [82–84]. However, acquired immunodeficiencies are far more common than hereditary predisposition. Furthermore, the causative nature of the specific gene defects has been less well-defined. Recently, a single variant in the caspase recruitment domain-containing protein 9 (*CARD9*) gene has been documented in Miniature Schnauzer dogs [85] (manuscript in preparation UG).

The CARD9 molecule is a multi-functional signaling protein and is essential in autonomous innate host defense against a variety of fungal species, including *Candida albicans*, *Aspergillus fumigatus*, or *Cryptococcus neoformans* and intracellular bacterial pathogens such as *Listeria monocytogenes* and *M. tuberculosis* [86, 87]. The CARD9 signaling pathway plays a pivotal role in autonomous innate host defense against *M. tuberculosis* [86]. Granulocytes from CARD9-deficient mice failed to produce IL-10 after infection and were not able to control bacterial replication, whereas T cell response appeared unaffected [86]. To date, similar effects of *CARD9* variants in humans have not been observed in association with mycobacterial infections. Genotyping showed that Miniature Schnauzer homozygous for the *CARD9* variant gene always developed systemic mycobacteriosis caused by *M. avium*. This is an autosomal recessively inherited trait, and a common ancestor of diseased dogs has been found dating back to the late 1980s. While over 100 Miniature Schnauzers have been found to have developed mycobacteriosis over the past three decades, a precision medicine approach including screening for this variant offers not only a precise diagnosis but also detection for carriers to avoid the production of any predisposed offspring in the breed. *Mycobacterium avium* is not the only opportunistic NTM that is present in the environment; and the reason why MAH infections are predominating remains unknown and might suggest a host-MAH-specific interaction.

The other breed with a genetic predisposition to MAC-associated mycobacteriosis is the Basset Hound. This unique susceptibility presents similarly but seems to be less common than in Miniature Schnauzers, and the underlying cause still needs to be elucidated. While tuberculosis due to MTBC infections in the gray wolves has been described, mycobacteriosis due to NTM has so far not been reported, further supporting the innate resistance of canidae against MAC except those with hereditary or acquired immunodeficiencies. Inbreeding does not raise the risk of new allele variants, but it can increase homozygosity of deleterious autosomal recessive traits as seen with the *CARD9* variant in Miniature Schnauzers [88]. The engagement and collaboration of researchers, veterinary clinicians, and dog breeders are therefore crucial for the further prevention of this fatal infection and to understand the underlying mechanisms of MAH invasion.

Diagnosis and Prevention

Ante-mortem diagnosis of a mycobacterial infection can be challenging, especially in cases where only internal organs are affected. Tuberculin skin tests (TST) for diagnosis of tuberculosis have been applied in dogs but has been shown to be of unreliable clinical utility and should not be used [20, 21, 89]. Currently, there are no validated commercial in vitro assays such as serological or whole-blood tests that can measure the immune reactivity of infected or sensitized dogs specifically against mycobacteria. With the interferon-gamma (IFN-γ) release assays (IGRA), the amount of released IFN-γ after antigen-specific stimulation can be quantified. The IGRA can be performed using purified protein derivative (PPD) from MTBC (e.g., *M. tuberculosis* or *M. bovis*), and avian PPD (*M. avium*) as antigens, or more specific stimulants such as early secretory antigenic target 6 kDa (ESAT-6) and culture filtrate protein 10 kDa (CFP-10). Commercial collection tubes coated on the inner surface with the necessary stimulants enabling immediate exposure of viable lymphocytes to specific mycobacterial antigens are now available [90]. Different IGRAs have been successfully used in domestic dogs and other canidae to detect immunological sensitization to MTBC [20, 90, 91]. Similarly, IGRAs for the early diagnosis of Johne's disease caused by MAP showed promising results in farm ruminants [65, 92, 93]. The ubiquitous nature of MAH impedes the implementation of an IGRA assay for canine *M. avium* detection, as it may merely reflect exposure to MAH and would not be informative about the current infection and disease status of the tested dogs.

Fine needle aspiration or biopsy specimens from cutaneous lesions and enlarged lymph nodes can readily be obtained in veterinary practice. In the presence of acid-fast bacteria after Ziehl-Neelsen staining and/or histopathological finding such as caseating granulomatous lesions, a mycobacterial infection can be suspected. However, the only definitive means to correctly identify the observed mycobacteria is by cultures or molecular methods. Due to the potential zoonotic risk and in cases where treatment is appropriate, it is important to identify the involved *Mycobacterium* sp. and perform antimicrobial susceptibility testing after mycobacterial isolation. However, mycobacterial cultures should only be performed by established specialized laboratories.

Techniques to identify rapid- and slow-growing mycobacteria depend on the laboratory infrastructure, equipment, expertise, and specimen to be tested. Traditional biochemical tests, specific reverse hybridization–based line-probe commercial kit assays, sequence analysis of selected housekeeping genes, and MALDI-TOF MS are valid methods currently used alone or in combination. Molecular genetic techniques, in particular specific gene or whole genome sequencing, allow for a better discrimination than traditional culture and biochemical methods, resulting in the recent

description of a myriad of new species. The 16S rRNA gene, coding for a component of the 30S small subunit of the prokaryotic ribosome, is the most common gene used for identification and phylogeny of bacteria [94]. Sequence analysis of the 16S rRNA gene has become a cornerstone of mycobacterial classification and taxonomy because of its discriminatory power and the availability of comprehensive freely accessible databases. However, because of the high genetic similarity of certain closely related mycobacteria, 16S rRNA gene analysis does not provide the necessary resolution capacity. For instance, the four subspecies of *Mycobacterium avium* are characterized by identical 16S rRNA gene sequences.

Beside the fundamental phenotypic features, such as mycobactin J dependency of MAP and MAS, additional markers for the differentiation of MAC species and *M. avium* subspecies have been described and should be applied. These include insertion sequences (IS), e.g., IS*901* present in MAA and MAS, IS*900* found in MAP and IS*Mav6*, which is a genetic variant of the original bird-type IS*901*, and a marker associated with virulent MAH strains [49••]. Another discriminating gene for *M. avium* subspecies is *hsp65*, particularly its 3′-region, which has been shown to contain subspecies-specific signatures [95]. When specifically analyzed, dogs with MAC mycobacteriosis are predominantly infected with MAH.

In Miniature Schnauzer dogs, the increased susceptibility to MAC infection and diagnosis of mycobacteriosis can be simply predicted even prior to exhibiting disease by showing homozygosity for the breed-specific *CARD9* variant by PCR testing. In this regard, the proactive screening of Miniature Schnauzers used in breeding programs is reducing the number of MAC susceptible dogs carrying this deleterious variant from the breed. No DNA test is currently available for testing the genetic susceptibility to MAC infections in Basset Hounds [85].

Management

Due to the high zoonotic risk of MTBC members, dogs diagnosed with tuberculosis should not be treated and humanely euthanized. Contact to dogs with TB should be restricted and exposed people, particularly those with immunodeficiency and immunosuppression, should be advised to contact their primary care physician and/or infectious disease specialist to undergo appropriate diagnostic investigations and potentially treatment. Although interspecies transmission of NTM has not been unequivocally described, the zoonotic potential of affected animals with any *Mycobacterium* sp. should not be underestimated. The minimal infective dose of most NTM species for human beings is yet to be determined, and infected dogs expose their potentially immunocompromised owners and other people to a far higher infection pressure even before they are showing obvious clinical signs. Among the members of the MAC, only *M. vulneris* has been specifically associated with a possible transmission between dogs and humans, as *M. vulneris* was cultured from an infected dog bite wound of a 42-year-old woman [96].

Because of the potential zoonotic risk and increasing interactions between human and companion animals, immunologically compromised people who are in close contact with NTM-infected dogs should be referred to their primary care physicians. According to the available literature, diagnostics and therapy of NTM infections depend on the mycobacterial (sub-) species, localized or systemic disease manifestation, as well as the host defense status. Any treatment attempts should be discouraged in breeds with an immunodeficiency.

Localized or disseminated cutaneous infections such as pyogranulomatous dermatitis and panniculitis with or without systemic clinical signs seem to have a favorable prognosis after prolonged antimicrobial therapy in dogs other than Miniature Schnauzers or Basset Hounds [79•, 97, 98]. Because isolation and susceptibility testing of mycobacteria is usually protracted for slowly growing mycobacteria, initial empiric antimycobacterial therapy is often necessary to impede the spread to adjacent tissue compartments [99]. The combination of antimycobacterial agents effective against slowly growing NTM, such as rifampicin and clarithromycin, combined with newer generation fluoroquinolones, e.g., moxifloxacin or pradofloxacin, has been used in isolated cases [32••, 100]. Doxycycline, macrolide, or fluoroquinolone as monotherapy is a common practice in veterinary medicine but may well not be effective even in immunocompetent dogs.

The experience from the literature shows that once the disease is disseminated, MAC mycobacteriosis is progressive and affected animals die or have to be euthanized regardless of any treatment attempts.

Infection Sources for *Mycobacterium avium*

Because of the ubiquitous nature of MAH in soil and water, different possible transmission routes, the broad host range, unspecific clinical signs, and long incubation period (weeks to months) of the disease, MAC infections in dogs represent epidemiological challenges (Fig. 1). The source and time of infection remain generally unknown and, thus, retrospective contact tracing is impractical. Whether infected animals, especially pets with close contact to their owners, play a role in the epidemiology of human mycobacteriosis caused by *M. avium* is still to be defined.

Noteworthy, only a low degree of homology between MAH isolates from Japanese human patients and local pigs was observed, whereas isolates from European human patients and from pigs in Japan and Europe were highly similar [53, 61•, 62•, 101, 102]. However, the source and time of MAC infection remains in most human patients and dogs unknown due to the long incubation period until overt disease occurs. Interregional or even international travel is commonly done for show dogs, pet purchases,

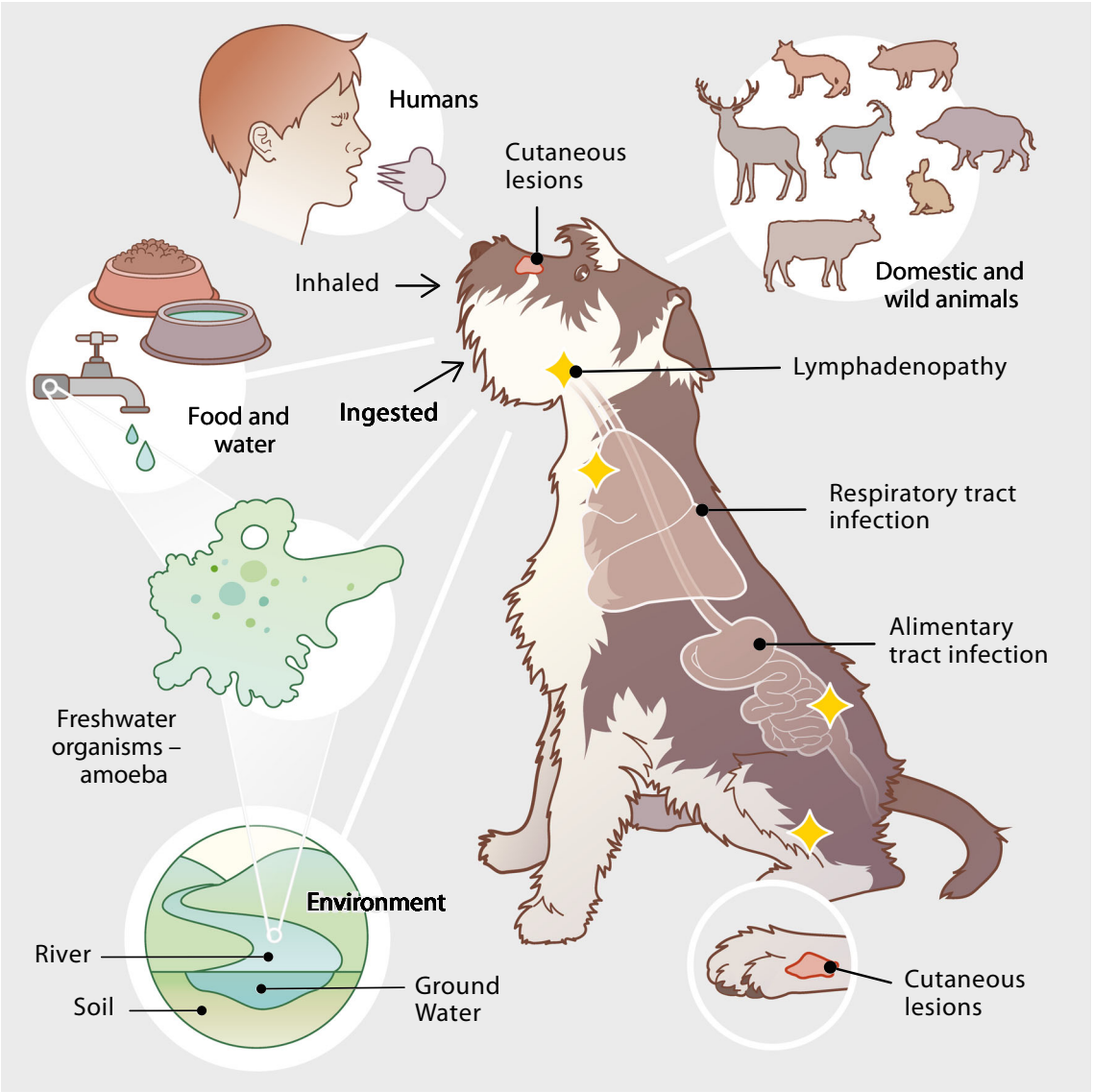

Fig. 1 Infection sources of *Mycobacterium avium* subsp. *hominissuis* (MAH) for dogs and common anatomical locations of mycobacteriosis; note frequently disseminated manifestation. *Mycobacterium avium* subsp. *hominissuis* is widely distributed in soil, ground- and tap water. While various mammals belong to the host range of MAH and potentially spread the mycobacteria into their living environment, birds are not likely to be a source of infection. Moreover, free-living organisms such as amoebae harbor MAH and may act as vehicles for the mycobacteria. Yellow stars represent the most commonly enlarged lymph nodes

breeding, and even for shelters. Whether MAC-infected dogs, especially pets with close contact to (immunocompromised) humans, play a role in infecting people remains to be determined.

A commonly discussed infection source for humans is aerosolized water particles such as those generated from showerheads. *M. avium* has been identified from biofilms of showerheads in the USA using a culture-independent approach [103], and bathroom's colonization with MAC organisms originating from infected patients has been hypothesized in Japan [104]. The possibility that MAC transfers from the patient to their bathroom, and that tap water may not be the source of infection, has been corroborated by studies from Japan, showing that NTM were rare or not detectable in bathroom samples from control patients and kitchen tap water [104, 105]. In Germany, MAH was recovered in dust and soil by culturing indoor and outdoor samples. Interestingly, MAH was significantly more often isolated from locations with close human contact such as indoor home dust and biofilms from sanitation facilities compared with countryside samples containing water, biofilm, and soil [106]. The MAC member *M. chimaera* was found in heater-cooler devices used in cardiac surgery leading to worldwide invasive nosocomial infections in humans [107, 108].

Free-living amoebae are common unicellular eukaryotes living in soil, brackish water, and potable water distribution systems [109] including hospital water systems [110]. They feed mainly via phagocytosis and inactivation of ubiquitous bacteria

and other microorganisms. However, many mycobacteria are known to be resistant against their phagocytosis and are able to reside unharmed and even multiply within the amoebal exocyst [111–114]. Thereby, mycobacteria can persist under adverse environmental conditions and against disinfectants, e.g., chlorine used for reduction of water-borne pathogens [115, 116]. Therefore, amoebal cysts are probable vehicles for opportunistic pathogens such as MAC, which can rapidly exit the cyst once ingested by mammals or further colonize the patient's environment. These findings suggest marked geographic differences, possibly influences by socio-economic aspects such as water supplies systems, local diets, and cooking habits.

Conclusions

Mycobacteriosis caused by MAC in an emerging disease in humans and dogs. While acquired immunodeficiencies prevail in human patients, a genetic predisposition to MAC in two breeds is responsible for most reported cases in dogs. Synergistic efforts from microbiologists, clinicians, infectious disease specialists, and breeders applying the One Health approach are necessary to improve our understanding of canine mycobacteriosis and assure animal and human health. Dogs with CARD9 deficiency may prove to be an appropriate animal model to study the host-microbe interactions and efficacy of treatments.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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- • Of importance
- •• Of major importance

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